Cinnamon-Derived Cinnamaldehyde and Empagliflozin Combination for HFD and STZ-induced Diabetic Nephropathy: An Experimental Study

Vinod A. Bairagi[1], Rupali S. Dongre[2], Jayesh H. Patil[2], Swapnil B. Jadhav[3], Yogesh S. Ahire[4*]

[1] Professor, Department of Pharmacology, KBHSS Trust's Institute of Pharmacy, Malegaon, Dist. Nashik, Maharashtra, India.

[2] Research Scholar, Department of Pharmacology, KBHSS Trust's Institute of Pharmacy, Malegaon, Dist. Nashik, Maharashtra, India.

[3]Assistant Professor, Department of Pharmacology, KBHSS Trust's Institute of Pharmacy, Malegaon, Dist. Nashik, Maharashtra, India.

[4] Associate Professor, Department of Pharmacology, KBHSS Trust's Institute of Pharmacy, Malegaon, Dist. Nashik, Maharashtra, India.

Address for correspondence:

Yogesh Suresh Ahire[*],

Associate Professor,

Department of Pharmacology,

KBHSS Trust's Institute of Pharmacy, Malegaon,

Dist. Nashik, Maharashtra, India,

yogesh.ahire2411@gmail.com.

Abstract

Background: Diabetic nephropathy (DN) represents a highly prevalent and extreme complication of type 2 diabetes mellitus (T2DM), which is caused by oxidative stress, inflammation, and loss of renal function in chronically hyperglycemic conditions. Available

treatments allow for slowing down the progression of the disease but fail to correct pre-existing pathology. An anti-diabetic drug combination with natural bioactives is an innovative avenue of treatment.

Objective: Research on the potential renoprotective effects of *cinnamon*-derived cinnamaldehyde, a natural anti-inflammatory product, combined with empagliflozin, which is a Selective SGLT2 inhibitor, in a rat model of T2DM-induced nephropathy via the high-fat diet (HFD) plus the low-dose streptozotocin (STZ) protocol.

Material and methods: Male Wistar rats were divided into five groups: normal control, HFD-STZ diabetic control, empagliflozin-treated, cinnamaldehyde-treated, and combination-treated. The HFD was used to induce diabetes by being administered. After 4 weeks, streptozotocin (35 mg/kg, i.p.) was administered. Hyperglycemia was confirmed after 4 weeks of DN induction in rats, and treatment continued for an additional 6 weeks. Biochemical values (blood glucose, urea, creatinine) and oxidative stress parameters (MDA, SOD) were assessed, along with renal histopathology.

Results: There was increased hyperglycemia and elevated serum urea/creatinine levels, with symptoms of lipid peroxidation and kidney tissue damage in HFD-STZ rats. Empagliflozin and cinnamaldehyde therapies, especially combined treatment, substantially alleviated glycemia, corrected indicators of renal dysfunction, reduced oxidative burden, and reduced histological renal injury compared with single treatment.

Conclusion: The present work establishes the prospects for the synergistic action of the empagliflozin-cinnamaldehyde complex in the therapy of HFD-STZ-induced diabetic nephropathy.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by persistently elevated blood sugar levels, and this can lead to unpleasant symptoms such as increased thirst, hunger, and frequent urination (Kumar *et al.,* 2020). It is a chronic disease characterised by hyperglycaemia, ultimately leading to microvascular (nephropathy) damage and macrovascular events such as myocardial infarction, cerebrovascular insults, and complications of peripheral vascular disease, including diabetic foot syndrome (Nauck *et al*., 2021).

Type 1 DM and Type 2 DM are the two types of DM. Type 1 DM is an autoimmune disorder that affects pancreatic cells, reducing or impairing insulin production. In contrast, type 2 DM results from impaired pancreatic beta-cell function, which impairs the individual's ability to use insulin (Padhi *et al*., 2020). Type 2 diabetes mellitus (T2DM) has rapidly become the most prevalent chronic disease worldwide and remains one of the major health challenges of the 21st

century (Da *et al*., 2013). Finally, the recent increase in the number of younger adults with T2D also contributes to the overall increase in T2D prevalence by prolonging their survival (Saeedi et al., 2019). The development of diabetes nephropathy, retinopathy, neuropathy, and macrovascular complications is attributable to advances in blood sugar regulation, and clinical and research efforts have been directed at elucidating the origins of diabetes and developing new treatments (Olefsky, 2001).

The denotation" diabetic nephropathy"(DN) describes a clinical condition characterized by intermittent albuminuria and a gradual drop in renal function, and it suggests the actuality of a distinctive pattern of glomerular compliance (Selby & Taal, 2020). Diabetic nephropathy is thought to result from disruptions in both metabolic and blood circulation processes, which are often affected in diabetes (Cao & Cooper, 2011).

The morphological features of diabetic kidney disease include tubular atrophy, mesangial expansion, interstitial fibrosis, thickening of the basement membrane, glomerular hypertrophy, and arteriole wall thickening (Kashihara *et al*., 2010). The "preclinical" stage of diabetic nephropathy, referred to as microalbuminuria, was first identified by researchers in the early 1980s (Parchwani & Upadhyah, 2012). As the condition progresses, it enters the "microalbuminuria" phase, defined by albumin-to-creatinine ratios of 2 to 28 mg of albumin per mmol of creatinine in an untimed urine sample, or albumin excretion between 30 and 300 mg per day (Dounousi *et al.*, 2015).

The pathogenesis of DN is driven by several factors, including hyperglycemia, oxidative stress, dyslipidemia, and advanced glycation end products (AGEs), which play important roles in both experimental animals and diabetic patients. Prolonged exposure to a high-fat diet leads to insulin resistance, and the development of diabetes occurs only in insulin-resistant HFD-fed rats following low-dose STZ, because the HFD-fed rats are already mildly hyperglycemic due to insulin resistance (Chen *et al*.,2019).

Angiotensin II (Ang-II), oxidative stress, and inflammation are key pathways and mediators implicated in the onset and progression of diabetic nephropathy (DN) and have recently received significant attention (Samsu, 2021). Macroalbuminuria is a better indicator of disease course than low-grade albuminuria, and Serum TNF-α receptor levels are currently the most promising biomarker, as they may predict the progression of CKD and ESRD in individuals with type 2 diabetes (Lim, 2014).

Cinnamaldehyde is known for its ability to modulate pro-inflammatory pathways by inhibiting NF-κB signaling, a critical regulator of inflammation. This inhibition reduces the production of key inflammatory mediators, such as TNF-α (Liao et al., 2012), and its anti-inflammatory

effects have been demonstrated in both in vitro and in vivo studies (Hancı *et al*., 2016). Sodium-glucose transport protein 2 (SGLT-2) inhibitors are the newest class of glucose-lowering drugs approved, which increase glycosuria by blocking glucose reabsorption in the proximal renal tubule (Nathan, 2015).

Empagliflozin is a highly effective SGLT2 inhibitor; its mechanism of action involves blocking SGLT2 activity, thereby enhancing urinary glucose excretion and lowering blood glucose concentrations, making it an important treatment option for managing hyperglycemia in type 2 diabetes (Fala, 2015).

Various reports have indicated that, as one might logically expect, combining an antioxidant with oral hypoglycemic agents may offer advantages over monotherapy (Kowluru, 2016). Combination treatments are more effective than monotherapy because of the combined effects of two treatments, such as empagliflozin with metformin, empagliflozin with ursolic acid, and cinnamaldehyde with eugenol.

Empagliflozin, an SGLT2 inhibitor, and Cinnamaldehyde, an antioxidant with anti-inflammatory effects, can be highly effective in preventing diabetic nephropathy when combined at low doses.

MATERIALS AND METHODS

Materials

An extract of cinnamon-derived cinnamaldehyde was obtained from Yukka Enterprises in India and stored at below 4 °C. STZ was obtained from Ottochemie Laboratories in Mumbai, India. USV Private Limited, Daman, provided a complimentary sample of metformin and empagliflozin. The study's biochemical estimation kits were from Meril Diagnostic Pvt. Ltd., India.

Experimental animals

Male Wistar rats (*Rattus norvegicus*), weighing between 200 and 250 g, were acquired from Lacsmi Bioform Pvt. Ltd., located in Pune 411027. The rats were fed a regular meal and provided an endless supply of water.

Study Design

The Institutional Animal Ethics Committee (IAEC) of the KBHSS Trust's Institute of Pharmacy, Malegaon, Nashik, approved the experimental protocol (KBH/IAEC/2024/12-03). Forty-two male rats in all were employed in the investigation. The experimental groups were given a high-fat diet (HFD) and a single intraperitoneal (i.p.) injection of streptozotocin (STZ) at a low dose of 35 mg/kg, dissolved in chilled citrate buffer (pH 4.5), after an overnight fast. The non-diabetic rats were given a standard chow diet. Blood glucose levels were monitored,

and rats with consistently elevated glucose were confirmed to be diabetic. For an extra four weeks (28 days), these diabetic rats were kept on the HFD with unlimited water to allow complications from diabetic nephropathy (DN) to develop. The animals were then randomly assigned to 7 groups (n = 6 per group) and treated for 6 weeks. As the normal control (NC), Group I received an oral dose of 1% sodium carboxymethyl cellulose (NaCMC). Group II served as the diabetic control (DC) and received 1% Na CMC without treatment. Group III received treatment with 200 mg/kg of the antidiabetic medication metformin in 1% NaCMC. Group IV received cinnamaldehyde (CN) at 20 mg/kg, and Group V received empagliflozin (EMPA) at 10 mg/kg. In comparison, Group VI (CE1) was administered a combination of cinnamaldehyde at 10mg/kg and empagliflozin at 5mg/kg. Finally, Group VII (CE2) received higher-dose combination therapy with cinnamaldehyde (20 mg/kg) and empagliflozin (10 mg/kg), with all treatments delivered orally in 1% Na CMC vehicle.

***In silico* docking study**

Molecular docking experiments for cinnamaldehyde and empagliflozin were performed using CBdock-II software. During pre-processing, water molecules were removed from the protein structure. After successful docking, the ligand-receptor complex with the lowest binding energy was identified.

The two-dimensional (2D) structure of empagliflozin and cinnamaldehyde was downloaded from PubChem and drawn in ChemDraw Ultra 8.0. This structure was subsequently converted to a three-dimensional (3D) model using Chem3D Ultra 8.0. The targeted proteins, such as 3ij7 (α-amylase), 5M2M (TNF-α), 1OEY (NADPH oxidase), 4YT1 (PPARγ), 5fzn (Nrf2), 3o8Y (lipooxygenase), 1GSH (Glutathione Synthetase), and 7vsi (SGLT2), were collected from the RSCB protein data bank in PDB format.

Biochemical and histopathological assessment of tissue homogenate (kidney)

Blood was collected by retro-orbital puncture, and serum was subsequently separated for biochemical analysis. Serum levels of glucose, triglycerides, total cholesterol, HDL-C, and LDL-C were measured using the Merilizer AutoQuant 100 Amara and commercial diagnostic kits (Meril Diagnostics Pvt. Ltd., India).

Rats were sacrificed, and the kidneys were immediately isolated. Kidney homogenate was prepared (0.1 M Tris-HCl buffer, pH 7.4). The supernatant was used to determine levels of LPO (Slater & Sawyer, 1971), GSH (Khan *et al*., 2014), SOD (Bagheri *et al*., 2024), CAT (Hadwan, 2016), and TNF-alpha using an ELISA kit (SciTesla). The remaining kidney was isolated, preserved in 10% formalin, and sent to SciTesla laboratory for histopathological examination.

Biochemical parameters from urine

At the end of the treatment, each rat was kept in a separate metabolic cage for a full day to collect urine. Numerous variable factors were measured directly from the urine samples. ELISA kits (SciTesla) were used to measure albumin and creatinine levels in urine samples according to the manufacturer's instructions.

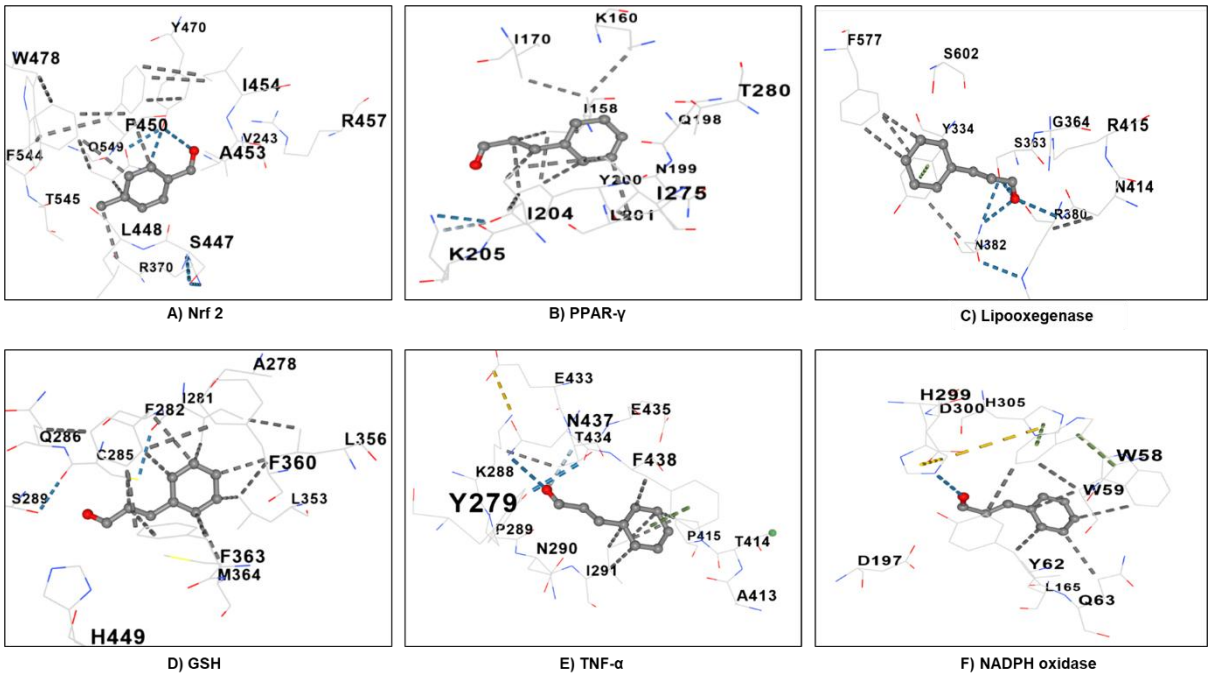
Statistical analysis

The mean ± standard deviation (SD) was used to display the data. Analysis was done using GraphPad Prism 8.4.2. Bonferroni's multiple-comparison test was used after a one-way ANOVA to assess the tissue and blood indicators.

RESULTS

In silico study

Figures 1 and 2 depict the ligand–protein interactions characterised by robust binding affinity. α-amylase, TNF-α, NADPH oxidase, PPARγ, Nrf2, lipoxygenase, Glutathione Synthetase, and SGLT2. Figure 1 demonstrates that cinnamaldehyde exhibits favourable binding affinities of -5.8, -5.2, -5.8, -6.4, -5.4, -5.6, -5.9, and -5.1 kcal/mol. In Figure 2, the binding energies (ΔG) of empagliflozin are recorded as -9.4, -9.3, -9.6, -8.7, -7.8, -11.8, -10.7, and -9.4 kcal/mol, respectively.



A) Nrf 2 B) PPAR-γ C) Lipooxegenase

D) GSH E) TNF-α F) NADPH oxidase

Figure 1: Molecular docking interaction of Cinnamaldehyde with protein targets associated with metabolic and haemodynamic pathways.

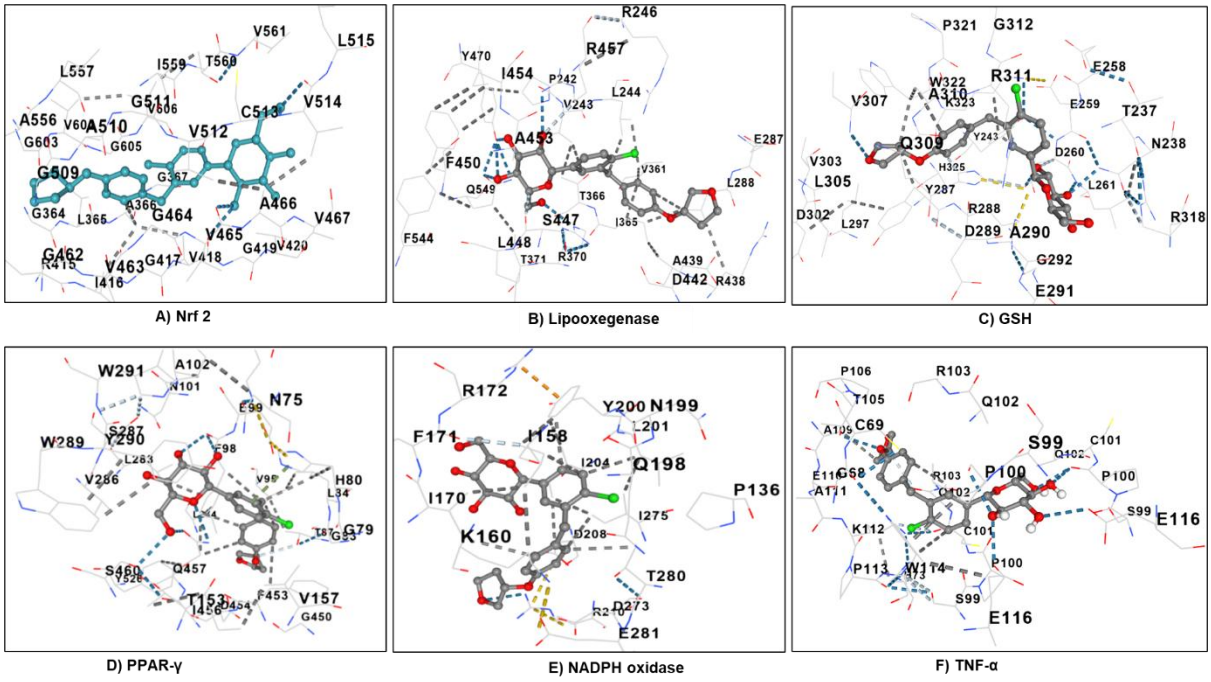


Figure 2: Molecular docking interactions of Empagliflozin with protein targets associated with metabolic and haemodynamic processes.

The Impact of Cinnamaldehyde in combination with Empagliflozin on Serum Glucose and Lipid Profile in Diabetic Rats

Diabetic control rats exhibit considerably higher serum glucose, cholesterol, triglycerides, and LDL-C levels, alongside markedly reduced HDL-C levels, compared with normal controls. The treatment group receiving Cinnamaldehyde with Empagliflozin (20+10 mg/kg) markedly improved hyperglycemia and lipid profile abnormalities compared with both the diabetic control and the groups treated with plain Cinnamaldehyde and Empagliflozin (Table 1).

Table 1. The Impact of Cinnamaldehyde in combination with Empagliflozin on Serum Glucose and Lipid Profile in Diabetic Rats

Parameter	Glucose level (mg/dL)	Serum Triglyceride (mg/dL)	Serum Cholesterol (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
NC	91 ± 5.329	82.33 ± 11.64	91.83 ± 4.271	36.13 ± 2.371	68.33 ± 3.34

DC	336.8 ± 2.881$^{###}$	178.2 ± 6.725$^{###}$	168.2 ±15.98$^{###}$	24.15±0.609$^{###}$	222.2±2.325$^{###}$
MET	142.8 ± 3.125***	97.66 ± 10.02***	97.83 ± 1.063***	34.83 ± 0.63***	94.56 ± 3.22***
EMPA	207.3 ± 7.494***	80.08 ± 10.39***	80.08 ± 10.39***	18.88 ± 3.09***	105.08 ± 0.49***
CN	226.5 ± 2.881***	123.9 ± 2.533***	123.9 ± 2.533***	24.79 ± 2.050***	115.9±1.800***
CE-I	117.5±3.507***, aa,bbb, ccc	104.9 ± 3.832***, aa, bb,c	113.9±3.114***, aa,b,c	30.20±1.472***, a,bbb, ccc	81.93±0.832***, aaa, bb, cc
CE-II	128.2 ± 2.317***, aaa,bbb, ccc	87.55±3.832***, aa,bbb, cc	99.21±9.942***, a,bbb, ccc	32.04±1.661***, a, bbb, ccc	83.55±1.032***, aaa,bbb, ccc

NC - Normal Control, DC – Diabetes Control, MET – Metformin, EMPA – Empagliflozin, CN – Cinnamaldehyde, CE-I – Cinnamaldehyde + Empagliflozin 1, CE-II – Cinnamaldehyde + Empagliflozin 2. Data are expressed as means ± standard deviation (n=6/group). The Bonferroni test for multiple comparisons was used to follow up on the one-way ANOVA. ###p<0.001 in comparison to normal control (NC); ***p<0.001 in comparison to diabetic control (DC); aaap<0.001 in comparison to Metformin (MET) group; bbbp<0.001 in comparison to Cinnamaldehyde (CN) group; cccp<0.001 in comparison to Empagliflozin (EMPA) group

Impact of Cinnamaldehyde in combination with Empagliflozin on serum albumin, creatinine, BUN, and Urine albumin and creatinine in diabetic rats

The diabetic group has increased serum creatinine and blood urea nitrogen levels, accompanied by diminished albumin levels. The combination led to a more pronounced decrease in creatinine and BUN levels, along with a significant elevation in albumin levels compared to cinnamaldehyde alone. In terms of urine creatinine and albumin levels observed with individual treatments, the CE-II group exhibited notable normalisation and enhancement in these parameters. This signifies that the combined therapy has improved nephroprotective efficacy (Table 2).

Table 2. Impact of Cinnamaldehyde in combination with Empagliflozin on serum albumin, creatinine, BUN, and renal albumin and creatinine in diabetic rats

Parameter	Serum albumin (g/dl)	Serum Creatinine (mg/dl)	Blood Urea Nitrogen (mg/dl)	Urinary albumin (g/dl)	Urinary Creatinine (mg/24hr)
NC	0.1583 ± 0.0.02	0.67 ± 0.6	24.33 ± 1.86	3.99 ± 0.17	1.67 ± 0.1
DC	1.385 ± 0.274$^{###}$	1.960 ± 0.070$^{###}$	49 ± 4.05$^{###}$	2.960± 0.1070$^{###}$	0.50 ± 0.050$^{###}$
MET	0.466 ± 0.0052***	1.116 ± 0.04***	25.83 ± 1.60***	4.66 ± 0.32***	0.9 ± 0.07***

EMPA	0.508 ± 0.039***	1.128 ± 0.06***	35.67 ± 1.86***	3.508 ± 0.539***	1.0 ± 0.06***
CN	0.429 ± 0.01871***	1.29 ± 0.03***	40.17 ± 1.94***	4.29 ± 2.533***	0.95 ± 0.06***
CE-I	0.229±0.01832***, aaa,bbb,c	0.89 ± 0.4***, aa,bbb,c	32.83 ± 1.72***, a,bbb, ccc	3.9 ± 0.832***, aaa,bbb, cc	1.15±0.09***, a,bbb, cc
CE-II	0.1800±0.012***, aaa,bbb, ccc	0.95 ±0.09***, aaa,bbb,c	33.83 ± 1.65***, aaa,bbb, ccc	4.95±0.032***, aaa,bbb, ccc	1.19±0.09***, aaa,bbb, cc

NC – Normal control, DC – Diabetes control, MET – Metformin, EMPA – Empagliflozin, CN – Cinnamaldehyde, CE-I – Cinnamaldehyde + Empagliflozin 1, CE-II – Cinnamaldehyde + Empagliflozin 2. Data are expressed as means ± standard deviation (n=6/group). The Bonferroni test for multiple comparisons was used to follow up on the one-way ANOVA. ###p<0.001 in comparison to normal control (NC); ***p<0.001 in comparison to diabetic control (DC); aaap<0.001 in comparison to Metformin (MET) group; bbbp<0.001 in comparison to Cinnamaldehyde (CN) group; cccp<0.001 in comparison to Empagliflozin (EMPA) group.

Impact of Cinnamaldehyde in combination with Empagliflozin on oxidative stress indicators and pro-inflammatory cytokine TNF-α levels in the kidneys of diabetic rats.

Diabetic control rats showed elevated levels of LPO and TNF-α, along with significantly reduced levels of GSH, SOD, and CAT compared with normal controls. Treatment with Cinnamaldehyde and Empagliflozin (20 + 10 mg/kg) effectively mitigated oxidative stress abnormalities and TNF-α levels compared with the diabetes control and the usual Cinnamaldehyde and Empagliflozin-treated group (Figure 3).

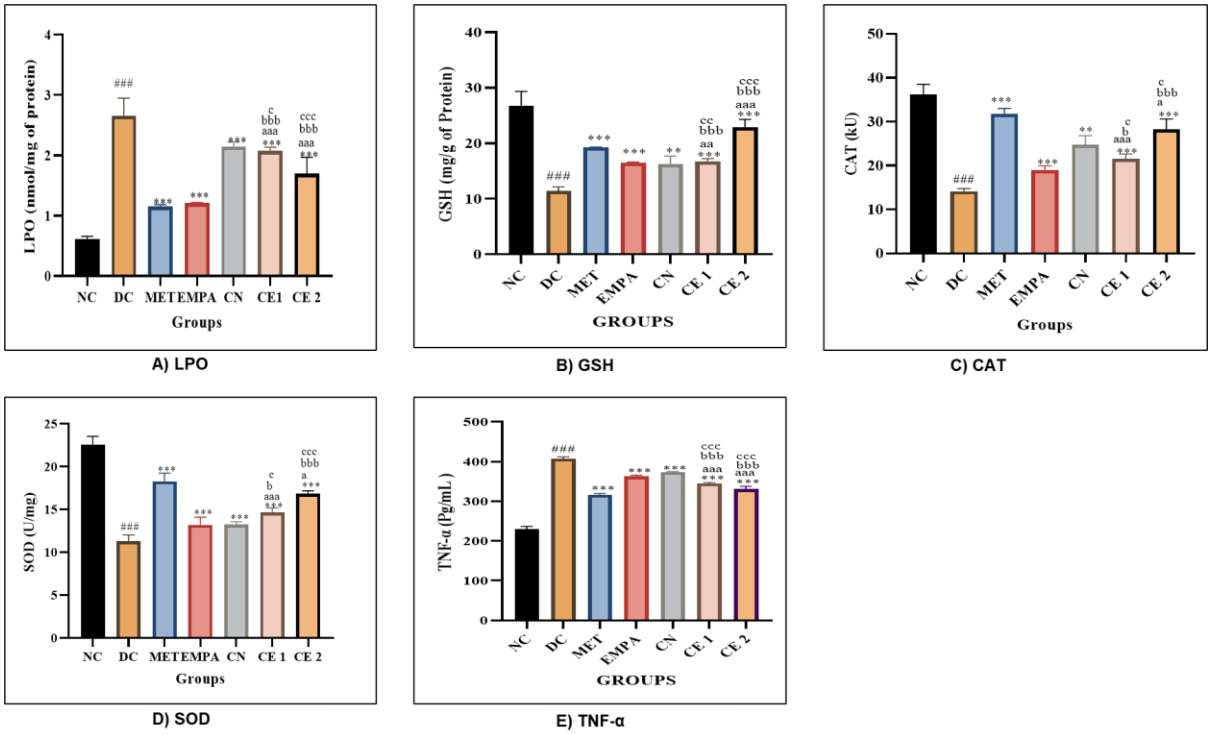


Figure 3. Impact of Cinnamaldehyde combined with Empagliflozin on oxidative stress indicators and pro-inflammatory cytokine TNF-α levels in the kidneys of diabetic rats. Graphical data are presented as means ± standard deviation (n=6/group). One-Way ANOVA succeeded by Bonferroni adjustment for multiple comparisons. ###p<0.001 in comparison to normal control (NC); ***p<0.001 in comparison to diabetic control (DC); aaap<0.001 in comparison to Metformin (MET) group; bbbp<0.001 in comparison to Cinnamaldehyde (CN) group; cccp<0.001 in comparison to Empagliflozin (EMPA) group.

Histopathological evaluation of cinnamaldehyde and empagliflozin in diabetic rat kidneys:

Histopathological evaluation of renal tissue from the non-diabetic (NC) group demonstrated no major abnormalities, with normal glomerular and tubular architecture. Conversely, kidneys from the diabetic control group showed mild to moderate nephropathic lesions, including cytoplasmic vacuolation, tubular dilatation, tubular atrophy, and expansion of Bowman's space, indicative of characteristic features of diabetic nephropathy. Rats with the empagliflozin-cinnamaldehyde combination demonstrated significant improvement in nephropathic

alterations. The severity and breadth of kidney lesions were significantly reduced compared with the diabetes control group.

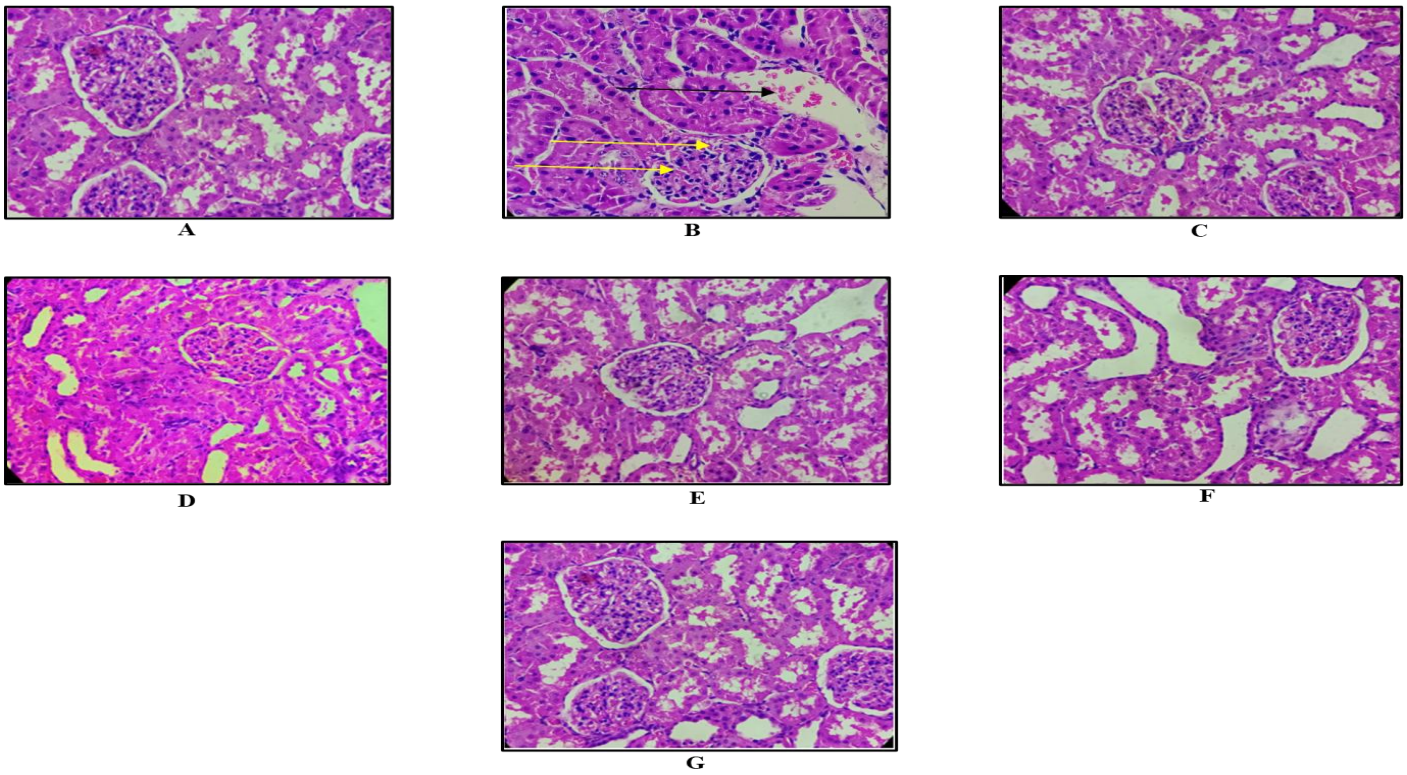


Figure 4: Histopathological evaluation of A) Group I (Normal Control), B) Group II (Diabetic Control), C) Group III (Standard), D) Group IV (Cinnamaldehyde), E) Group V (Empagliflozin), F) Group VI (Cinnamaldehyde+Empagliflozin-I), G) Group VII (Cinnamaldehyde+Empagliflozin-II)

DISCUSSION

In our study, we discovered synergistic nephroprotective effects when empagliflozin, a selective SGLT2 inhibitor, was combined with cinnamaldehyde, a bioactive phytochemical with potent antioxidant properties, in an HFD-STZ-induced diabetic rat model. Our results reveal that this combination therapy not only significantly mitigates renal dysfunction as evidenced by marked improvements in serum creatinine, blood urea nitrogen, urinary albumin excretion, and creatinine clearance but also robustly suppresses oxidative stress and inflammatory responses within renal tissue.

The induction of diabetes in experimental models using streptozotocin (STZ) is well established. STZ is a glucose analog that is selectively taken up by pancreatic β-cells through

the low-affinity GLUT2 transporter (Lenzen, 2007). Once inside the cell, STZ acts as an alkylating agent, damaging DNA and inhibiting β-cell O-GlcNAcase, which removes O-GlcNAc from proteins (Tesch & Allen, 2007). This results in irreversible O-glycosylation, cellular dysfunction, and ultimately β-cell apoptosis, leading to insulin deficiency and a state of insulin-dependent diabetes mellitus (Tesch & Allen, 2007). A hallmark feature of pancreatic β-cells is their remarkable ability to couple glycolysis with mitochondrial oxidation, allowing for efficient glucose sensing and insulin secretion (Oleson *et al*., 2019).

A substantial body of evidence implicates the overexpression of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 in the initiation and progression of diabetic kidney injury (Lim & Tesch, 2012). TNF-α and IL-6, key cytokines that mediate both acute and chronic inflammation, are associated with all-cause morbidity and mortality in the general population and in predialysis and dialysis patients (Lee *et al*., 2019).

Chronic hyperglycemia further exacerbates renal injury by increasing the formation of advanced glycation end products (AGEs), which activate their receptor (RAGE) and downstream signaling pathways, promoting inflammation and fibrosis. Hyperglycemia also induces oxidative stress by increasing the production of reactive oxygen species (ROS) and the accumulation of free radicals in renal tissues (Forbes & Cooper, 2013). A fall in the insulin-to-glucagon ratio causes increased production of glucose by the liver (basal hyperglycemia), whereas the absolute decrease in plasma insulin concentration or action reduces glucose utilization in peripheral tissues (postprandial hyperglycemia) (Giugliano *et al*.,2008).

Most of the glucose filtered by the glomerulus is reabsorbed in the proximal tubule of the kidney, a process primarily mediated by the sodium-glucose co-transporter 2 (SGLT2). By inhibiting this co-transport mechanism, glucose reabsorption is reduced, leading to increased glucose excretion in the urine (glucosuria) and lower blood glucose levels (Kalra, 2014).

Moderate ROS-mediated damage can be reversed; excessive ROS generation overwhelms antioxidant defenses, leading to irreversible cellular damage and acceleration of pathological states, including DN (Jin *et al*., 2023). Antioxidant enzymes such as SOD, glutathione peroxidase (GSH-Px), catalase (CAT), glutathione reductase (GR), and paraoxonase are critical for neutralizing ROS. Markers of oxidative damage, such as MDA and protein carbonyls, reflect the extent of lipid peroxidation and protein oxidation, respectively, and are exacerbated in diabetes (Bhatia *et al*., 2003). SOD catalyzes the oxidation/reduction/conversion of superoxide radicals (O_2^-) to molecular oxygen and H_2O_2 (Xie *et al*., 2016). The resulting

oxidative stress is closely linked to mitochondrial dysfunction, activation of pro-apoptotic pathways, and the progression of nephron loss.

Oral administration of cinnamaldehyde has been shown to promote regeneration of islet cells and stimulate insulin secretion by protecting β-cells from free radical insults (Kumar *et al*., 2012). This is achieved in our studies on the assessment of antioxidant enzymes by preventing their glycation, including SOD, CAT, and GSH, suggesting that natural products with antioxidant properties may support pancreatic islet regeneration and insulin secretion.

Nrf2 is a redox-sensitive transcription factor that regulates the expression of over 250 genes involved in cellular defense against oxidative stress (Chen *et al*., 2018). Cinnamaldehyde supplementation is associated with reduced oxidative stress and attenuated induction of the profibrotic mediator transforming growth factor-beta (TGF-beta), cyclin-dependent kinase inhibitor p21, and extracellular matrix proteins in diabetic kidneys (Zheng *et al*., 2011). Empagliflozin not only improved diabetic myocardial structure and function but also decreased myocardial oxidative stress, thereby ameliorating myocardial fibrosis, a finding further confirmed to be correlated with activation of the Nrf2/HO-1 signaling pathway (Shi *et al*., 2022). Molecular docking studies elucidate the binding affinities and interactions of cinnamaldehyde and empagliflozin with the Nrf2/Keap1 complex or related signaling proteins, supporting their potential to activate the Nrf2 pathway and confer antioxidative protection in diabetic complications.

Cinnamaldehyde, which demonstrated multiple antidiabetic and renoprotective mechanisms, enhances GLUT4 translocation to the plasma membrane in adipose and muscle tissues in a dose-dependent manner, thereby improving glucose uptake and insulin sensitivity (Shen *et al*., 2010). Cinnamaldehyde also modulates peroxisome proliferator-activated receptor (PPAR) pathways, which play key roles in regulating insulin resistance, adipogenesis, and lipid metabolism (Medagama, 2015). Molecular docking studies indicate a strong binding affinity of cinnamaldehyde and related compounds for PPARγ, suggesting a potential antidiabetic mechanism of action.

In diabetic rats, cinnamaldehyde has been shown to increase glucose uptake, improve insulin sensitivity in tissues, and ameliorate diabetic nephropathy. It also inhibited the accumulation of AGEs, RAGE, and carboxymethyl lysine (CML), and downregulated transcription of pro-inflammatory and profibrotic mediators such as IL-1β, TNF-α, NF-κB, and TGF-β (Fatima *et al.*, 2024).

Beyond glycemic control, empagliflozin has demonstrated pleiotropic benefits, including reductions in blood pressure, improvements in lipid profiles, and lowering of serum uric acid, likely through its antioxidative effects (Choobkar *et al*., 2024). Empagliflozin reduced oxidative stress, as demonstrated by lower malondialdehyde (MDA) levels and increased activities of antioxidant enzymes such as SOD, while suppressing apoptosis and improving renal function.

Assessment of renal function in the STZ-induced DN model relies on markers such as serum creatinine (SCr), blood urea nitrogen (BUN), urinary albumin excretion rate (UAER), and creatinine clearance (CCr), which reflect glomerular filtration and overall kidney health (Zhang *et al*., 2017). Creatinine is a breakdown product of creatine phosphate in muscle, and its clearance rate from blood to urine (CCr) correlates with glomerular filtration rate; therefore, CCr can be used as an indicator for kidney function (Zhang *et al*., 2017). Our findings show increased plasma BUN and creatinine, and urinary albumin, indicating progression of nephrotoxicity in rats in the disease group. Elevated urinary albumin excretion is a sensitive indicator of glomerular injury and is strongly associated with the progression of chronic kidney disease in diabetes (Matboli *et al*., 2017). Combination therapy with empagliflozin and cinnamaldehyde significantly reduced urinary albumin excretion in the study, suggesting glomerular protection and enhanced function.

Conclusion

This study shows that the combination of empagliflozin and cinnamaldehyde provides greater protection against HFD-STZ–induced diabetic nephropathy than either treatment alone. The combined therapy markedly enhanced glycaemic control, reinstated renal function markers, and diminished oxidative stress and inflammation. Histopathological findings confirmed significant restoration of kidney architecture. The combined effects of SGLT2 inhibition and antioxidant properties resulted in improved renoprotection. This combination presents a promising therapeutic strategy for the management of diabetic nephropathy and requires additional preclinical and clinical investigation.

Future scope:

Future research must investigate the long-term safety, efficacy, and optimal dosing of the empagliflozin–cinnamaldehyde combination in diabetic nephropathy. Research should also examine its impact on additional diabetes-related complications, such as cardiovascular, neurological, and hepatic damage. Molecular and genomic assessments may elucidate

underlying mechanistic pathways. Furthermore, extending this research to larger animal models and ultimately clinical trials may confirm its translational applicability for human use.

References:

1. Kumar, R., Saha, P., Kumar, Y., Sahana, S., Dubey, A., & Prakash, O. (2020). A review on diabetes mellitus: type1 & Type2. *World Journal of Pharmacy and Pharmaceutical Sciences*, *9*(10), 838-850. https://doi.org/10.20959/wjpps202010-17336

2. Nauck, M. A., Wefers, J., & Meier, J. J. (2021). Treatment of type 2 diabetes: challenges, hopes, and anticipated successes. *The lancet Diabetes & endocrinology*, *9*(8), 525-544.https://doi.org/10.1016/s2213-8587(21)00113-3

3. Padhi, S., Nayak, A. K., & Behera, A. (2020). Type II diabetes mellitus: a review on recent drug-based therapeutics. *Biomedicine & Pharmacotherapy*, *131*, 110708.https://doi.org/10.1016/j.biopha.2020.110708.

4. Da Costa Mousinho, N. M., van Tonder, J. J., & Steenkamp, V. (2013). In vitro anti-diabetic activity of Sclerocarya birrea and Ziziphus mucronata. *Natural product communications*, *8*(9), 1934578X1300800924. https://doi.org/10.1177/1934578x1300800924.

5. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., ... & IDF Diabetes Atlas Committee. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes research and clinical practice*, *157*, 107843.https://doi.org/10.1016/j.diabres.2019.107843.

6. Olefsky, J. M. (2001). Prospects for research in diabetes mellitus. *Jama*, *285*(5), 628-632.https://doi.org/10.1001/jama.285.5.628.

7. Selby, N. M., & Taal, M. W. (2020). An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. *Diabetes, Obesity and Metabolism*, *22*, 3-15. https://doi.org/10.1111/dom.14007.

8. Cao, Z., & Cooper, M. E. (2011). Pathogenesis of diabetic nephropathy. *Journal of diabetes investigation*, *2*(4), 243-247. https://doi.org/10.1111/j.2040-1124.2011.00131.x.

9. Kashihara, N., Haruna, Y., K Kondeti, V., & S Kanwar, Y. (2010). Oxidative stress in diabetic nephropathy. *Current medicinal chemistry*, *17*(34), 4256-4269.https://doi.org/10.2174/092986710793348581.

10. Parchwani, D. N., & Upadhyah, A. A. (2012). Diabetic nephropathy: Progression and pathophysiology. *Int J Med Sci Public Health*, *1*(2), 59-70. https://doi.org/10.5455/ijmsph.2012.1.59-70.

11. Dounousi, E., Duni, A., Leivaditis, K., Vaios, V., Eleftheriadis, T., & Liakopoulos, V. (2015). Improvements in the management of diabetic nephropathy. *The review of diabetic studies: RDS*, *12*(1-2), 119.https://doi.org/10.1900/rds.2015.12.119.

12. Chen, H. W., Yang, M. Y., Hung, T. W., Chang, Y. C., & Wang, C. J. (2019). Nelumbo nucifera leaves extract attenuate the pathological progression of diabetic nephropathy in high-fat diet-fed and streptozotocin-induced diabetic rats. *journal of food and drug analysis*, *27*(3), 736-748.https://doi.org/10.1016/j.jfda.2018.12.009.

13. Samsu, N. (2021). Diabetic nephropathy: challenges in pathogenesis, diagnosis, and treatment. *BioMed research international*, *2021*(1), 1497449.https://doi.org/10.1155/2021/1497449.

14. Lim, A. K. (2014). Diabetic nephropathy–complications and treatment. *International journal of nephrology and renovascular disease*, 361-381.https://doi.org/10.2147/ijnrd.s40172.

15. Liao, J. C., Deng, J. S., Chiu, C. S., Hou, W. C., Huang, S. S., Shie, P. H., & Huang, G. J. (2012). Anti-inflammatory activities of Cinnamomum cassia constituents in vitro and in vivo. *Evidence-Based Complementary and Alternative Medicine*, *2012*(1), 429320. https://doi.org/10.1155/2012/429320.

16. Hancı, D., Altun, H., Çetinkaya, E. A., Muluk, N. B., Cengiz, B. P., & Cingi, C. (2016). Cinnamaldehyde is an effective anti-inflammatory agent for treatment of allergic rhinitis in a rat model. *International journal of pediatric otorhinolaryngology*, *84*, 81-87.https://doi.org/10.1016/j.ijporl.2016.03.001.

17. Nathan, D. M. (2015). Diabetes: advances in diagnosis and treatment. *Jama*, *314*(10), 1052-1062.https://doi.org/10.1001/jama.2015.9536.

18. Kowluru, R. A. (2016). Role of Oxidative Stress in Epigenetic Modification of Matrix Metalloproteinase-9 in the Development of Diabetic Retinopathy. *Free Radical Biology and Medicine*, *100*, S176.. https://doi.org/10.1016/j.freeradbiomed.2016.10.464.

19. Slater, T. F., & Sawyer, B. C. (1971). The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. General features of the systems used. *Biochemical Journal*, *123*(5), 805-814. https://doi.org/10.1042/bj1230805.

20. Khan, R., Upaganlawar, A. B., & Upasani, C. (2014). Protective effects of Dioscorea alata L. in aniline exposure-induced spleen toxicity in rats: a biochemical study. *Toxicology International*, *21*(3), 294. https://doi.org/10.4103/09-6580.155371.

21. Bagheri, R. B., & Khaki, A. A. (2024). Effects of carvacrol on hormonal and biochemical blood factors related to diabetes in diabetic adult rats induced by streptozocin. *Medical Journal of Tabriz University of Medical Sciences*, *46*(2), 136-144.https://doi.org/10.34172/mj.2024.020.

22. Hadwan, M. H. (2016). New method for assessment of serum catalase activity. *Indian Journal of science and technology*, *9*(4), 1-5. https://doi.org/10.17485/ijst/2016/v9i4/80499.

23. Lenzen, S. (2008). The mechanisms of alloxan-and streptozotocin-induced diabetes. *Diabetologia*, *51*(2), 216-226. https://doi.org/10.1007/s00125-007-0886-7.

24. Tesch, G. H., & Allen, T. J. (2007). Rodent models of streptozotocin-induced diabetic nephropathy (Methods in Renal Research). *Nephrology*, *12*(3), 261-266. https://doi.org/10.1111/j.1440-1797.2007.00796.x.

25. Oleson, B. J., Broniowska, K. A., Yeo, C. T., Flancher, M., Naatz, A., Hogg, N., ... & Corbett, J. A. (2019). The role of metabolic flexibility in the regulation of the DNA damage response by nitric oxide. *Molecular and Cellular Biology*, *39*(18), e00153-19.https://doi.org/10.1128/mcb.00153-19.

26. Lim, A. K., & Tesch, G. H. (2012). Inflammation in diabetic nephropathy. *Mediators of inflammation*, *2012*(1), 146154. https://doi.org/10.1155/2012/146154.

27. Lee, H. C., Shiou, Y. L., Jhuo, S. J., Chang, C. Y., Liu, P. L., Jhuang, W. J., ... & Lee, A. S. (2019). The sodium–glucose co-transporter 2 inhibitor empagliflozin attenuates cardiac

fibrosis and improves ventricular hemodynamics in hypertensive heart failure rats. *Cardiovascular diabetology*, *18*(1), 45. https://doi.org/10.1186/s12933-019-0849-6.

28. Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiological reviews*, *93*(1), 137-188. https://doi.org/10.1152/physrev.00045.2011.

29. Giugliano, D., Ceriello, A., & Esposito, K. (2008). Glucose metabolism and hyperglycemia. *The American journal of clinical nutrition*, *87*(1), 217S-222S. https://doi.org/10.1093/ajcn/87.1.217s.

30. Kalra, S. (2014). Sodium glucose co-transporter-2 (SGLT2) inhibitors: a review of their basic and clinical pharmacology. *Diabetes Therapy*, *5*(2), 355-366. https://doi.org/10.1007/s13300-014-0089-4.

31. Fala, L. (2015). Jardiance (empagliflozin), an SGLT2 inhibitor, receives FDA approval for the treatment of patients with type 2 diabetes. *American health & drug benefits*, *8*(Spec Feature), 92. https://pmc.ncbi.nlm.nih.gov/articles/PMC4665046/.

32. Jin, Q., Liu, T., Qiao, Y., Liu, D., Yang, L., Mao, H., ... & Zhan, Y. (2023). Oxidative stress and inflammation in diabetic nephropathy: role of polyphenols. *Frontiers in immunology*, *14*, 1185317. https://doi.org/10.3389/fimmu.2023.1185317.

33. Bhatia, S., Shukla, R., Madhu, S. V., Gambhir, J. K., & Prabhu, K. M. (2003). Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy. *Clinical biochemistry*, *36*(7), 557-562. https://doi.org/10.1016/s0009-9120(03)00094-8.

34. Xie, R., Zhang, H., Wang, X. Z., Yang, X. Z., Wu, S. N., Wang, H. G., ... & Ma, T. H. (2017). The protective effect of betulinic acid (BA) diabetic nephropathy on streptozotocin (STZ)-induced diabetic rats. *Food & function*, *8*(1), 299-306. https://doi.org/10.1039/c6fo01601d.

35. Chen, Q., Tao, J., & Xie, X. (2018). Astaxanthin promotes Nrf2/ARE signaling to inhibit HG-induced renal fibrosis in GMCs. *Marine drugs*, *16*(4), 117. https://doi.org/10.3390/md16040117.

36. Zheng, H., Whitman, S. A., Wu, W., Wondrak, G. T., Wong, P. K., Fang, D., & Zhang, D. D. (2011). Therapeutic potential of Nrf2 activators in streptozotocin-induced diabetic nephropathy. *Diabetes*, *60*(11), 3055-3066. https://doi.org/10.2337/db11-0807.

37. Shi, P., Zhan, Z., Ye, X., Lu, Y., Song, K., Sheng, F., ... & Yin, P. (2022). The antioxidative effects of empagliflozin on high glucose-induced epithelial-mesenchymal transition in peritoneal mesothelial cells via the Nrf2/HO-1 signaling. *Renal failure*, *44*(1), 1529-1543. https://doi.org/10.1080/0886022x.2022.2118066.

38. Shen, Y., Fukushima, M., Ito, Y., Muraki, E., Hosono, T., Seki, T., & Ariga, T. (2010). Verification of the antidiabetic effects of cinnamon (Cinnamomum zeylanicum) using insulin-uncontrolled type 1 diabetic rats and cultured adipocytes. *Bioscience, biotechnology, and biochemistry*, *74*(12), 2418-2425. https://doi.org/10.1271/bbb.100453.

39. Medagama, A. B. (2015). The glycaemic outcomes of Cinnamon, a review of the experimental evidence and clinical trials. *Nutrition journal*, *14*(1), 108. https://doi.org/10.1186/s12937-015-0098-9

40. Fatima, N., Khan, M. I., Jawed, H., Qureshi, U., Ul-Haq, Z., Hafizur, R. M., ... & Shazly, G. A. (2024). Cinnamaldehyde ameliorates diabetes-induced biochemical impairments and AGEs macromolecules in a pre-clinical model of diabetic nephropathy. *BMC Pharmacology and Toxicology*, *25*(1), 85. https://doi.org/10.1186/s40360-024-00811-0.

41. Choobkar, S., Alilou, M., Mahmoudvand, G., & Rouzbahani, A. K. (2024). Evaluation of the Effects of Empagliflozin on Serum Levels of Triglycerides and Cholesterol in Patients with Type 2 Diabetes Mellitus and Hypertriglyceridemia: A Prospective Study. *Iranian journal of diabetes and obesity*. https://doi.org/10.18502/ijdo.v16i4.17287.

42. Zhang, S., Xu, H., Yu, X., Wu, Y. I., & Sui, D. (2017). Metformin ameliorates diabetic nephropathy in a rat model of low-dose streptozotocin-induced diabetes. *Experimental and therapeutic medicine*, *14*(1), 383-390. https://doi.org/10.3892/etm.2017.4475.

43. Peng, J., Song, X., Yu, W., Pan, Y., Zhang, Y., Jian, H., & He, B. (2024). The role and mechanism of cinnamaldehyde in cancer. *Journal of Food and Drug Analysis*, *32*(2), 140. https://doi.org/10.38212/2224-6614.3502.

44. Matboli, M., Eissa, S., Ibrahim, D., Hegazy, M. G., Imam, S. S., & Habib, E. K. (2017). Caffeic acid attenuates diabetic kidney disease via modulation of autophagy in a high-fat

diet/streptozotocin-induced diabetic rat. *Scientific reports*, *7*(1), 2263. https://doi.org/10.1038/s41598-017-02320-z.